

Serial No.: 09/900,509

Filed: July 5, 2001

On page 24, immediately preceding the claims, please insert the enclosed text entitled "SEQUENCE LISTING".

REMARKS

The specification has been amended to include a Sequence Listing and proper reference to the sequences therein. Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

Entry of this amendment is respectfully requested. The amendments are made in adherence with 37 C.F.R. § 1.821-1.825. This amendment is accompanied by a floppy disk containing the above named sequence, SEQUENCE ID NUMBERS 1-3 in computer readable form, and a paper copy of the sequence information. The computer readable sequence listing was prepared through use of the software program "PatentIn" provided by the PTO. The information contained in the computer readable disk is identical to that of the paper copy. This amendment contains no new matter. Applicant submits that this amendment, the accompanying computer readable sequence listing, and the paper copy thereof serve to place this application in a condition of adherence to the rules 37 C.F.R. § 1.821-1.825.

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Please direct any calls in connection with this application to the undersigned at (415)
781-1989.

Respectfully submitted,

FLEHR HOHBACH TEST
ALBRITTON & HERBERT LLP

Dated: 12/19/01

Four Embarcadero Center
Suite 3400
San Francisco, CA 94111-4187
Telephone: (415) 781-1989


Richard F. Trecartin, Reg. No. 31,801

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Paragraph beginning at page 8, line 6, has been amended as follows:

—As should be understood, the list of transcription factors is known to the artisan. The members of each class and subclass are specifically incorporated by reference. Binding sites for transcription factors are well known in the art. A database of transcription factors is available through TRANSFAC - The Transcription Factor Database at <http://transfac.gbf.de/TRANSFAC/>. Preferred transcription factors include NF- κ B, ETS, STAT, p53, Ap-1 family, steroid hormone and related families. In a preferred embodiment, NF- κ B binding sites are used. A variety of sites can be used including the consensus site 5'GGGRNYYYCC3' (SEQ ID NO:1) described in Chen and Ghosh, Oncogene 1999, 18:6845-6852. —

Paragraph beginning at page 12, line 6, has been amended as follows:

— Each pool is assayed for the ability to activate a transcription factor-dependent reporter in a transient transfection assay in tissue culture cells. Pools with the desired regulatory activity (either stimulatory or inhibitory) are further subdivided and screened until a single cDNA is isolated with the desired activity. As a control, positive pools are screened for the ability to regulate a mutant version of the reporter, in which the NF- κ B binding sites have been mutated. Positive pools which do not regulate the mutant reporter are determined to be specific. In a preferred embodiment, the NF- κ B-responsive promoter has two copies of the κ B site (5'-GGGGACTTTCC-3' (SEQ ID NO:2)). The mutant reporter is identical except that the two NF- κ B sites have been mutated to (5'-ATCCACTTTCC-3' (SEQ ID NO:3)). Eventually, a single cDNA is obtained which will encode a gene product that can regulate the activity of the transcription factor of study. This

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individual clone is then sequenced to identify the cDNA it contains and the gene product it expresses.—

Paragraph beginning at page 19, line 8, has been amended as follows:

— 293T cells were maintained in DME supplemented with 10% fetal calf serum, 100 U/ml of penicillin and streptomycin, and 2mM glutamine in humidified 5% CO₂ at 37°C. Cells were plated at 9 x 10⁴/well in 24-well dishes 24 hr before transfection by the calcium phosphate method. A total of 372 ng of DNA was transfected, including 2 ng of pCSK-LacZ, 20 ng of the Ig κ ₂-IFN-LUC reporter, and 350 ng of pool DNA. The Ig κ ₂-IFN LUC reporter contains two copies of the Ig κ ₂ κ B site (5'-GCGGACTTTCC-3' (SEQ ID NO:2)) upstream of the interferon- β minimal reporter (-55 to +19) driving luciferase expression (Fujita, T. et al., *Cell* 49:357-67 (1987)). pCSK-lacZ vector constitutively expresses β -galactosidase and is unaffected by NF- κ B activity.—

Paragraph beginning at page 20, line 16, has been amended as follows:

— For efficient and economical expression screening, a reporter assay for NF- κ B activation that is quantitative and highly sensitive was employed. In this assay, pool DNA was transiently transfected into 293T cells with the Ig κ ₂-IFN-LUC reporter, which contains two copies of the Ig κ κ B site (5'-GCGGACTTTCC-3' (SEQ ID NO:2)) upstream of the interferon- β minimal promoter (-55 to +19) (Fujita, T., et al., *Cell* 49:357-67 (1987)) driving luciferase expression. For normalization for transfection efficiency and extract recovery, the transfection includes the pCSK-lacZ vector (Condie, B.G., et al., *Mol Cell Biol* 10:3376-85 (1990)) which constitutively expresses β -galactosidase and is unaffected by NF κ B activity. To maximize the number of cDNAs that could be assayed in a transfection, the complexity was determined (number of cDNAs per pool) which would allow reliable detection of a single active clone in a mixture of cDNAs. Pilot experiments using TRAF2, an adapter protein in the TNF α pathway (Rothe, M., et al., *Science* 269:1424-7 (1995)), suggested that a pool complexity of 100 cDNAs would allow detection of

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molecules possessing 3-fold lower specific activity than TRAF2 in this assay (data not shown). The sensitivity of detection of luciferase and β -galactosidase activities allowed us to scale down the size of the transfection and to minimize the amount of pool DNA required.—

Paragraph beginning at page 21, line 7, has been amended as follows:

— Three secondary screens were applied. First, the NF- κ B dependence of a pool's activity was tested by comparing its fold induction on the Ig κ ₂-IFN-LUC reporter to that on the MUT-IFN-LUC reporter, which contains mutations in the Ig κ κ B motifs (5'-ATCCACTTTCC-3' (SEQ ID NO:3)). Second, the specific activities which might function upstream of the IKK complex were tested by assessing their activity in the presence of the IKK β K44A kinase-dead dominant negative. Third, each κ B specific positive pool was tested in the presence of kinase dead TBK1 (K38A), an IKK related kinase which we recently identified (Pomerantz, J.L. & D. Baltimore, *Embo J* 18:6694-704 (1999)) (see also (Tojima, Y., et al., *Nature* 404:778-82 (2000))). Examples of these secondary screens are shown in FIG. 2. Of the 41 positive pools, 34 were found to be dependent on the κ B sites for activity. Each of these specific pools was found to be inhibited by cotransfection with the IKK β K44A, and one pool (pool 178) was also inhibited by cotransfection with TBK1 K38A (FIG. 2).—

On page 24, immediately preceding the claims, the enclosed Sequence Listing was added to the text.